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The ultrastructure of alcoholic liver disease: A review and analysis of 100 biopsies

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Summary. Liver biopsies from 100 patients with alcoholic liver disease of various grades of severity were examined by light and electron microscopy. A comprehensive account of ultrastructural morphology is presented. The organellar changes were variable both in nature and intensity. The most consistent ultrastructural changes, irrespective of disease severity, were dilatation of endoplasmic reticulum and fat accumulation. The former is the EM counterpart of hepatocyte swelling at the light microscope level. Collagen deposition was detected earlier and more accurately by electron microscopy. A physical relationship between Mallory bodies and intermediate filament was also detected. As is the case with light microscopy, if cellular, stromal and organellar changes are considered independently, some may be regarded as typical, but none as pathognomonic of alcoholic liver disease.

Key words: Ultrastructure-Alcohol-Liver

Introduction

The ultrastructure of alcoholic liver disease (ALD) in humans has been the subject of numerous investigations. Since the early 1960's, publications dealing with the subject include series of a few patients (Reppart et al., 1963; Porta et al., 1965; Rubin and Lieber, 1975), series in which there is no mention of the category of alcoholic liver damage (Biava, 1964; Schaffner and Popper, 1963a; Kiessling et al., 1965), studies mainly dealing with a given organelle (Svoboda and Manning, 1964; Kiessling and Pilstrom, 1971; Rubin, 1971; Burns et al., 1972; Oudea et al., 1973; Bruguera et al., 1977; Sternlieb, 1979; Chedid et al., 1980; Wu et al., 1984) or structure (Schaffner and Popper, 1963a; Biava, 1964; Flax and Tisdale, 1964; Smuckler, 1968; Sternlieb et al., 1971; Yokoo et al., 1972; Sternlieb and Quintana, 1977; Orrego et al., 1979; Schaff and Lapis, 1979; French, 1983) and studies of cells other than hepatocytes (Schaffner et al., 1963b; Rudolph et al., 1979; Minato et al., 1980; Nakano et al., 1982; Okanove et al., 1983). Some of these reports relied upon findings in volunteers (Lane and Lieber, 1966; Rubin and Lieber, 1967).

This study presents a comprehensive account of the ultrastructural changes found in 100 patients with ALD of various grades of severity.

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Materials and methods

One hundred patients investigated for ALD at the Liver Unit of the John Radcliffe Hospital during the past 9 years, were selected on the basis of having had a liver biopsy examined by light and electron microscopy (EM). Fifty-six patients were male and 44 female, aged between 22 and 79 years (mean age 52 years). All patients had a past history of heavy alcohol intake and clinical and biochemical evidence of liver disease. Percutaneous or operative liver biopsy was performed for diagnostic purposes. Variables such as diet, recent alcohol intake versus timing of biopsy, were not analysed in this study.

The larger portion of the biopsies was fixed in 10% formalin, embedded in paraffin, and sections stained with haematoxylin and eosin. All biopsies were also stained for identification of collagen, reticulin, iron. The histopathological diagnoses are given in Table 1.

For ultrastructural studies a portion of the sample was fixed in cold 4% buffered gluteraldehyde. Specimens were postfixed in 2% osmiun tetroxide, dehydrated and

embedded in plastic (Araldite, Epon or Emix). Semi-thin sections were examined by light microscopy and ultrathin sections examined from areas of light microscopic abnormality. Ultrathin sections were stained with lead citrate and uranyl acetate and examined by transmission electron microscopy. A retrospective analysis of the photographic prints taken largely at random was then made.

Results

In most biopsies, the low power EM morphology was in accordance with the diagnoses made by light microscopy (Table 1). In those specimens without significant distortion of lobular architecture, it was easy to identify the different constituents of the portal tracts. When fibrosis and nodular regeneration were present, it was difficult to distinguish portal tracts from fibrotic bands containing proliferated ductules and blood vessels.

Hepatocytes

The nuclei of viable hepatocytes showed no significant changes. Mitoses were scarce. When fat accumulation was conspicuous the nucleus was distorted and displaced towards the cell periphery. In alcoholic hepatitis and cirrhosis, necrotic hepatocytes showed a decrease in nuclear volume, condensation of karyoplasm and a variable degree of thickening of the nuclear membrane. The changes occurring in the cytoplasm in all forms of ALD, are detailed below.

Endoplasmic reticulum. In most biopsies, the cisternae of both rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER) were dilated (Figs. 1,2). This change varied from cell to cell and also within individual hepatocytes. Proliferation of the SER was also present but not invariably. In some cells it was impossible to be sure if SER or degranulated RER had proliferated because ribosomes and polyribosomes were found close by many apparently smooth ER channels.

Golgi apparatus. This was rarely present, despite careful searching. Therefore, the number of complexes in alcohol damaged hepatocytes may be diminished.

Mitochondria. The most significant and constant change involved this organelle (Fig. 3). Mitochondrial volume varied from extreme enlargement (megamitochondria) to subtle changes which were very difficult to interpret without formal morphometric analysis. In none of our cases were we able to find mitochondria which were equal in size to the nucleus. Variations in shape were particularly striking. Elongation in one of two planes was most often seen, resulting in an ovoid or even spindle configuration. Angulation was not infrequent. In some cases shape change was more extensive; this gave rise to mitochondria with a pleomorphic appeareance which do not fit any geometric shape. In a given cell, these changes were not universal. Normal mitochondria coexisted with the abnormal forms described (Fig. 2). The matrix was sometimes normal but more often showed increased density. Cristae were shortened, distorted or absent. In the altered mitochondria, electron lucent vacuoles, filamentous crystals or laminated inclusions were present (Fig. 3). Dense bodies were frequently larger than usual and haphazardly distributed.

Table 1. Histopathological diagnoses in 100 patients with alcoholic liver disease.

Fatty Liver	22
Fatty liver with fibrosis	2
Alcoholic hepatitis	22
Alcoholic hepatitis with fibrosis	12
Fibrosis	6
Cirrhosis	18
Cirrhosis with alcoholic hepatitis	13
Nonspecific morphology consistent with ALD	5

Lysosomes. They were increased when there was liver cell necrosis. They showed a variable appearance. In some instances, typical multivesicular bodies and autophagic vacuoles were prominent. In other instances they engulfed residual material of unknown nature. Lipofuscin was sometimes abundant. Siderosomes were conspicuous in cases with iron deposition. Lipolysosomes were exceptionally seen.

Peroxisomes. No significant changes were seen in this organelle.

Fat. Accumulation of fat in hepatocytes was a frequent ultrastructural finding in all categories of ALD (Fig. 4). No limiting membrane was detected and their size was variable. The EM equivalent of fatty cyst in conventional microscopy, was composed of fine droplets separated by a thin membrane presumably derived from the cell plasma membrane.

Glycogen. It was present in variable amounts. When depleted it was difficult to exclude inadequate preservation due to delayed fixation. When abundant it was uniformally distributed and exceptionally found as glycogen bodies.

Bile. In specimens with cholestasis, material considered to represent retained bile showed different appearances: granular, lamellar, crystalline or whorled. Only in a few instances could we find an expanded pericanalicular cytosol and/or electrondense material within the Golgi apparatus.

Mallory bodies (MBs). They were present in biopsies with alcoholic hepatitis alone or associated with cirrhosis and only in a small proportion of those in which they were detected by light microscopy. MBs were found by EM in two cases where they were not detected by light microscopy. MBs were composed of haphazardly oriented fibrils and were unrelated to any organelle (Fig. 3). In some cells small aggregates of fibrillar material were considered as early MBs or classic MBs cut tangentially. At the periphery of a few MBs there were small filaments with a diameter similar to intermediate filaments.

Cell membrane. In chronic ALD the sinusoidal surface of the liver cell membrane showed various degrees of distortion and atrophy of microvilli. In many cases these changes were related to an increased amount of collagen in the space of Disse. The lateral surfaces of the cell membrane in chronic ALD showed variable amounts

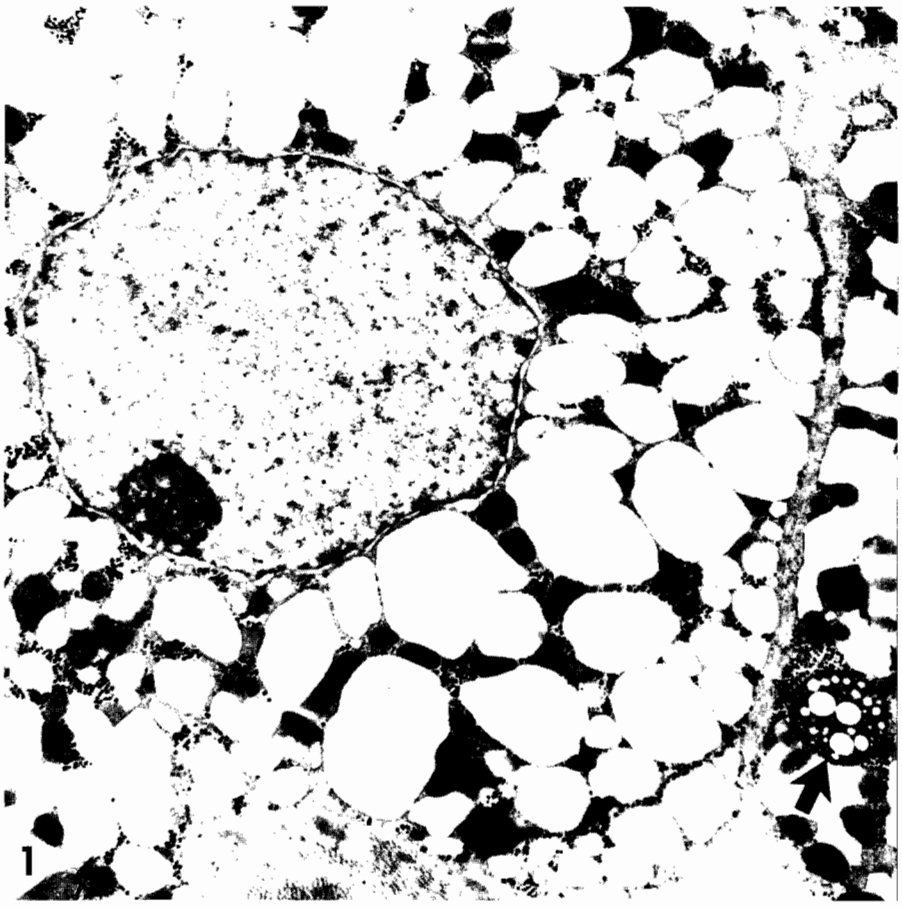

Fig. 1. Ballooned hepatocyte with marked dilatation of the ER. Mitochondria are uniform but show increased matrix density. The adjacent liver cell shows a microvesicular body (arrow). (Cirrhosis with alcoholic hepatitis). x 9,000

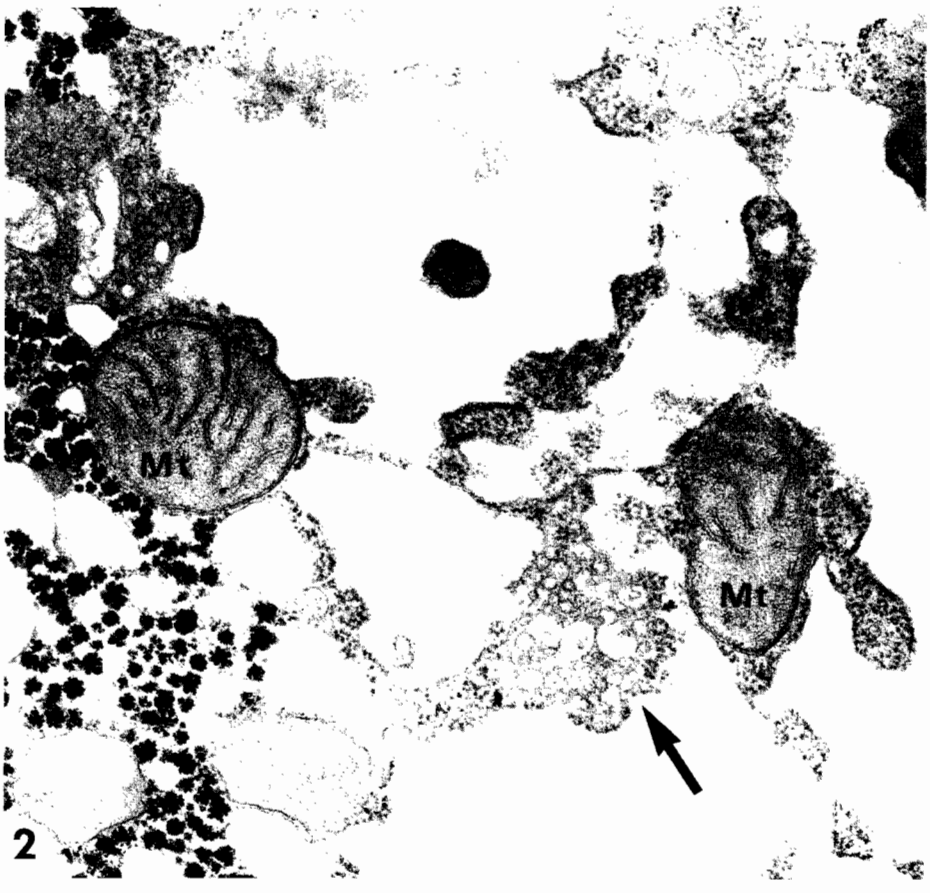

Fig. 2. Dilated ER with partially degranulated RER and a small portion of SER with microvesicular appearance (arrow). Mitochondria (Mt) are undremarkable. (Cirrhosis with alcoholic hepatitis). x 32,500

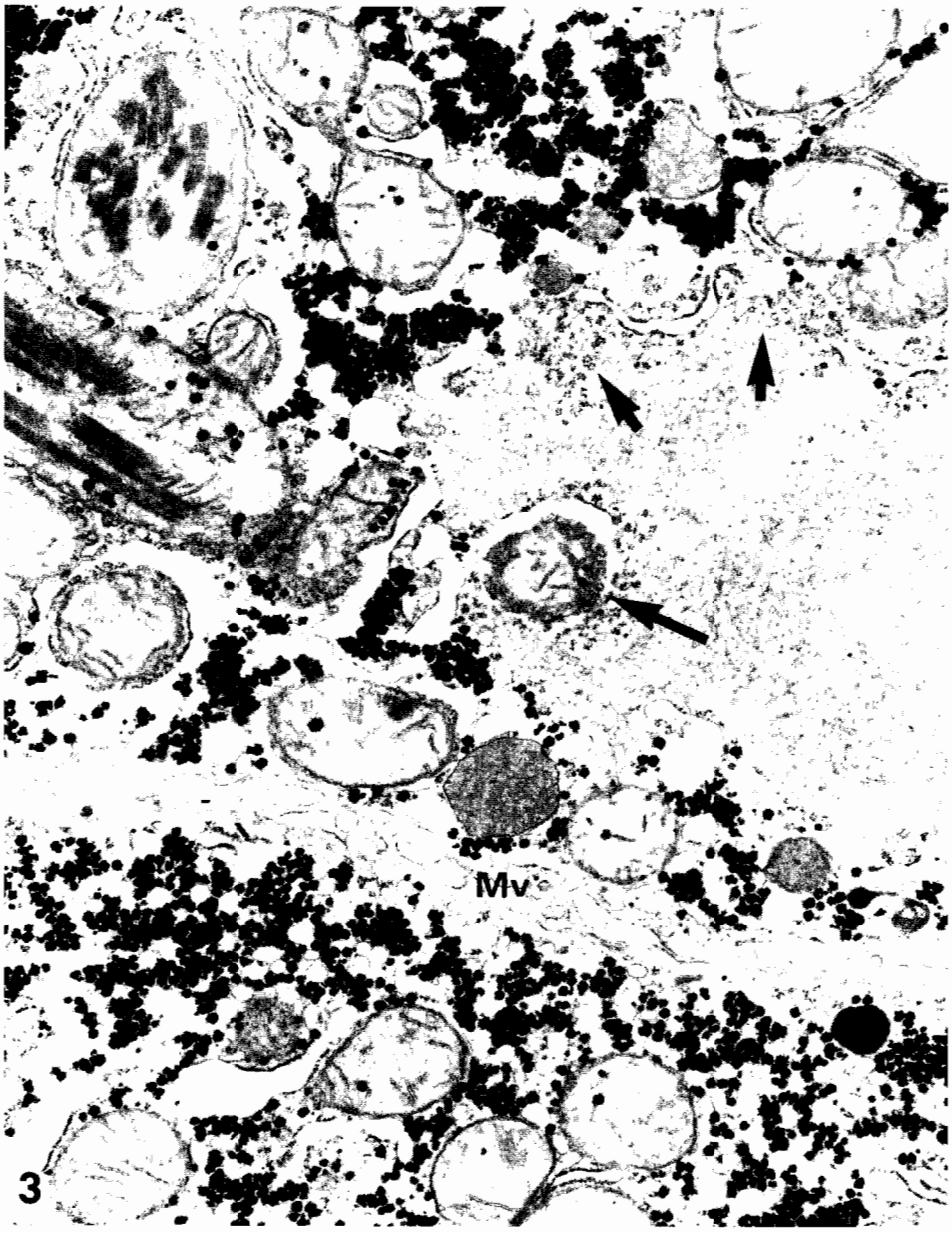

Fig. 3. Mallory body surrounding mitochondria (long arrow). Polyribosomes are at the periphery of this Mallory body. Mitochondira vary in size and shape. The larger ones contain paracrystalline inclusions and prominent dense bodies. The lateral surfaces of the liver cell membrane include microvilli (Mv). (Alcoholic hepatitis with fibrosis). x 19,600

of microvilli (Fig. 3). In contrast, when liver cell damage was marked, cytoplasmic bullae were found. Occasionally ectoplasmic condensation and increase in the density of the cell membrane was also apparent. In active cell damage these changes coexisted with disruption of the intercellular connections (desmosomes and junctional complexes) and with detachment of isolated hepatocytes from liver cell plates.

When cholestasis was present, bile canalicular microvilli were shortened and distorted. These changes coexisted with dilatation of the canalicular lumina and eventually with changes in the pericanalicular cytosol and Golgi apparatus (see above). Only rarely were junctional complexes disrupted.

Sinusoids and perisinusoidal space

Changes in sinusoids and Disse's space varied according to the type of ALD. In fatty liver the sinusoidal wall was usually unremarkable. In alcoholic hepatitis the changes varied according to the area examined. In necrotic foci, or adjoining areas, sinusoids contained inflammatory cells; Kupffer cells were enlarged and showed prominent phagocytic activity. Expanded sinusoidal spaces most often

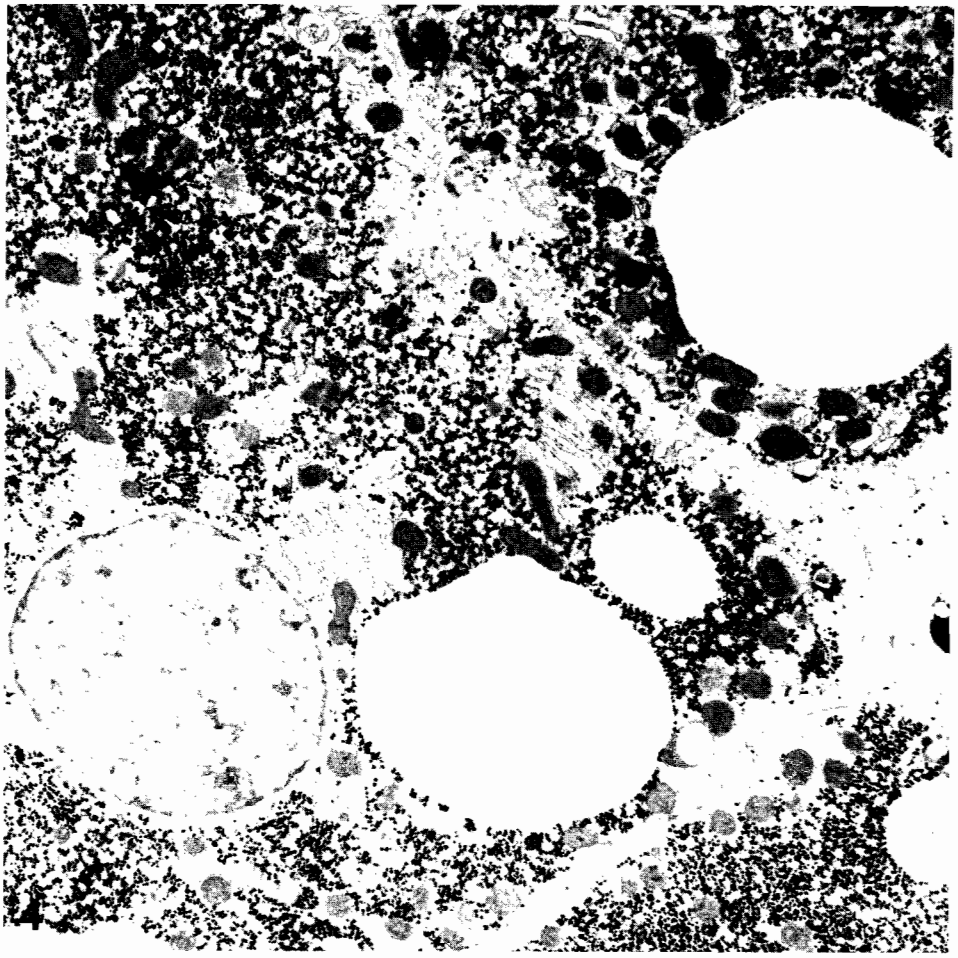

Fig. 4. Well demarcated lipid droplets devoid of a limiting membrane. (Alcoholic cirrhosis). x 6,000

contained leucocytes and macrophages. In contrast, when liver cells were ballooned, the sinusoids appeared narrowed. In most biopsies, irrespective of the severity of the disease process, there was an increased number of perisinusoidal cells. They were larger than normal and contained numerous lipid droplets, some indenting the nucleus (Fig. 5).

In areas distant from liver cell damage, there were only a few inflammatory cells, or none at all. Kupffer cells were conspicuous but with lesser amounts of phagocytozed material.

In chronic ALD, alcoholic hepatitis and fatty change only, there was an increase in collagen fibres in the space of Disse. This was especially so in cirrhosis. Sometimes, collagen extended between hepatocytes (Fig. 6). Fibroblasts were seldom found along the collagen bundles of the perisinusoidal spaces but when present, were near the central veins. A continuous basement membrane along sinusoids was seldom found.

Inflammatory infiltrates

Inflammatory cells were found in all types of ALD except fatty change only. In alcoholic hepatitis and cirrhosis with hepatitis, polymorphonuclear leucocytes and histiocytes (macrophages) were the predominant cell type. With milder inflammatory changes, lymphoid cells were more conspicuous but plasma cells were scarce.

Portal spaces and septa

Only a few portal spaces were examined in steatosis and alcoholic hepatitis, and no significant changes were detected in the bile ductules or the arterioles. The amount of collagen and the presence of inflammatory cells was variable. In fibrosis and cirrhosis, collagen appeared as closely packed fibres. In addition to fibroblasts, proliferated bile ducts and vessels were present. Myofibroblasts were only occasionally present.

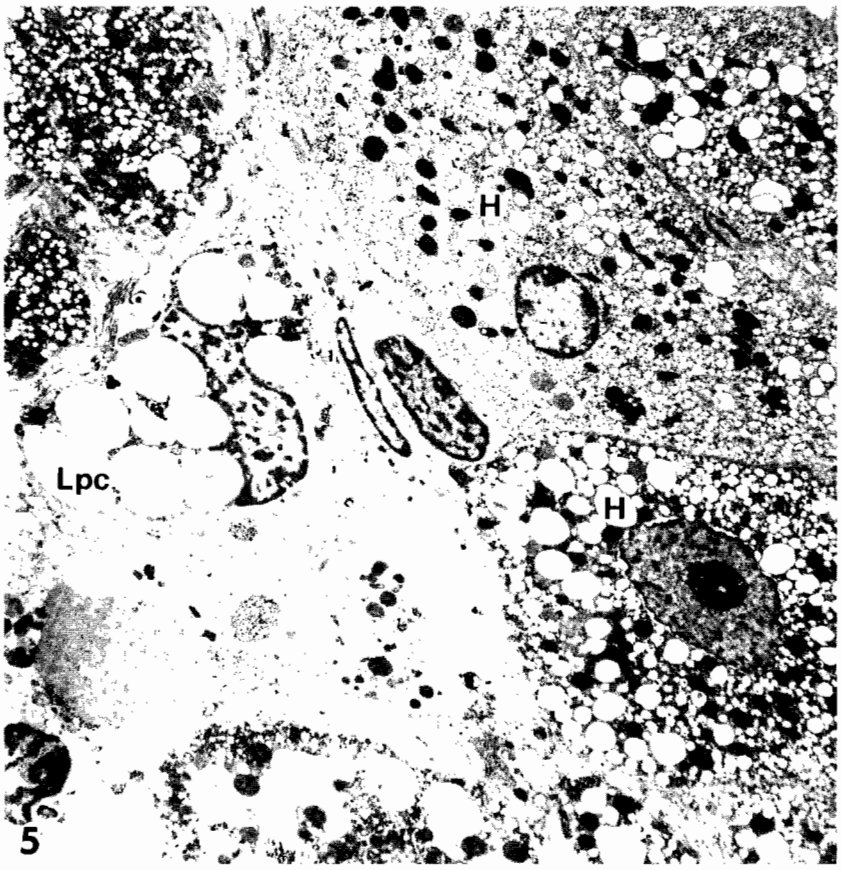

Fig. 5. An enlarged lipocyte (Lpc) contains numerous fat droplets, some indenting its nucleus. Hepatocytes (H) have vesiculated endoplasmic reticulum. (Alcoholic cirrhosis). x 5,000

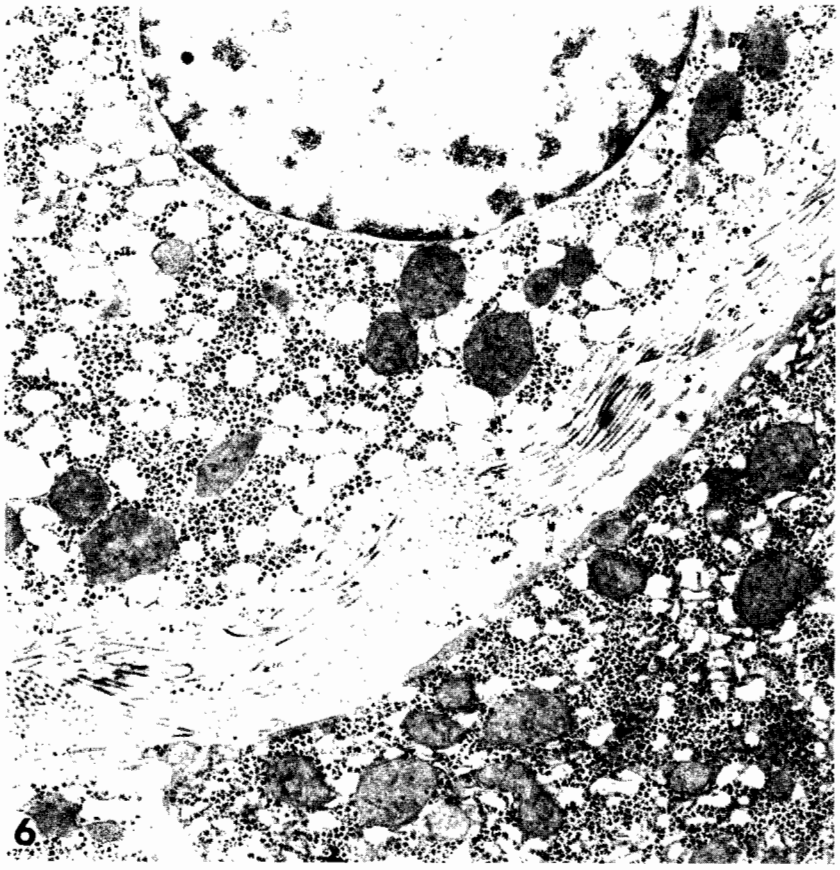

Fig. 6. Partial view of two hepatocytes with collagen bundles in between. The cell above, has slighted dilated ER. Glycogen is uniformly distributed. (Alcoholic hepatitis with fibrosis). x 16,500

Discussion

Following the first reports from Mount Sinai Hospital, New York (Popper and Schaffner, 1963; Schaffner and Popper, 1963a; Schaffner et al., 1963c) of ultrastructural findings in ALD, there have been at least 50 publications on the subject (Rubin and Lieber, 1968; Iseri and Gottlieb, 1971; Blendis et al., 1982). Some are reviews or descriptions of part of the spectrum of ALD. Many of these do not mention the number of patients (Shaffner et al., 1963c; Schaffner, 1971; Popper et al., 1981), while others deal with small series and only 13 reports contain more than 20 alcoholics (e.g. Oudea et al., 1968; Ma, 1972; Horvath et al., 1973b). Occasional series include previously published cases (Kiessling and Pilstrom, 1971; Gerber et al., 1973; Blendis et al., 1982). This report presents a comprehensive account of the ultrastructural changes in the largest collection of ALD biopsies examined by EM. In this discussion no attempt is made to distinguish between EM findings in the different forms of ALD because of sampling error implicit in any EM study.

The most consistent and typical ultrastructural abnormality in ALD is dilatation of ER. The type and degree of disturbance of the ER depend on the extent of hepatocellular damage (Winckler, 1971; Horvath et al., 1973b). In some cells it can remain intact or show a microvesicular configuration as the result of SER hypertrophy (Kiessling, 1968; Horvath et al., 1973a). When cisternae are dilated, they may appear empty (hydropic change only) or contain osmiophilic material (Kiessling, 1968; Rubin and Lieber, 1968). If damage is more marked, fragmented and degranulated profiles are seen (Schaffner et al., 1963c). Ribosomes can be encountered singly or as polyribosomes proximate to dilated ER. Some authors consider that this represents dislocation of ribosomes from RER (Kiessling, 1968). However, dilatation of apparent SER may represent either changes in SER per se or RER denuded of ribosomes (Kiessling, 1968). This study does not resolve this question.

Another constant EM finding in this series was mitochondrial abnormality. This took the form of mitochondrial enlargement, change in organellar shape, and the presence of crystalline, laminated or electronlucent inclusions in the mitochondrial matrix. Since the early descriptions of ultrastructural changes in ALD, mitochondrial alterations have been the subject of particular attention (Popper and Schaffner, 1963; Schaffner et al., 1963c; Reppart et al., 1963). Kiessling et al. (1965), observed a good correlation between the number of enlarged mitochondria and the degree of alcohol abuse. The following year alterations of mitochondria were reported in response to alcohol ingestion by normal subjects under balanced dietary conditions (Lane and Lieber, 1966). Since then, numerous contributions emphasise not only the increase in size and variations in shape, but the increase of matrix density and alterations in the number, length and configuration of the mitochondrial cristae. (Smuckler, 1968; Bruguera et al., 1977; Sternlieb, 1979). Attention is drawn to the appearance of paracrystalline inclusions and of dense granules, the latter more numerous and larger than normal. The review article by Sternlieb (1979) gives a detailed account of these changes.

In 1971, Kiessling and Pilstrom confirmed their previous observations that when mitochondria were only slightly enlarged, no other abnormalities could be elicited. In contrast, paracrystalline inclusions were present in enlarged ovoid or elongated mitochondria; as were parallel cristae. These changes were frequently present in the liver of patients with moderate or high alcohol intake.

Bruguera et al. (1977), demonstrated that the globular hyaline cytoplasmic inclusions of regular outline seen at light microscopy were megamitochondria; an observation previously made by Winckler in 1970. Spheroidal giant mitochondria had a homogeneous appearance, with a matrix density about the same as that of normal mitochondria. The elongated forms had a higher density and often showed crystalline inclusions. According to Horvarth et al. (1973a), large and spherical mitochondria are rarely observed in liver biopsies other than those from alcoholics.

Rubin (1971), using isocaloric substitution of alcohol for carbohydrates, showed in volunteers, that hepatic mitochondria were enlarged and disfigured, with breaks of the outer membrane, disorientation of cristae and paracrystalline inclusions. Oudea et al. (1973) in five alcoholics without clinical evidence of liver disease found that the mitochondrial density was increased as a ratio of "fat free" cytoplasmic volume and not of total cytoplasmic volume. This suggests that conspicuous changes, previously described, are more linked to severe forms of alcoholic hepatitis and not a constant feature of chronic alcoholism.

More recently, Wu et al. (1984), measured the size, number and surface membrane area of the mitochondria in ALD, and found that the volume density per cubic micrometer of cytoplasm, was not significantly increased. This may be explained by the exclusion of acute alcoholic hepatitis from the study, a finding which is also in agreement with that of Kock et al. (1977) in alcoholics without evidence of liver disease. A frequent finding in this study was the accumulation of nonmembrane bound fat in hepatocytes. In most reports in which fat accumulation is described, there is a consensus that there is variation in the size of fat droplets and in the absence of a limiting membrane (Porta et al., 1965; Uchida et al., 1983). When a membrane is seen, in the exceptional case, it is most likely SER filled with lipid (Ma, 1972). In liver cells with foamy degeneration, droplets are minute and numerous (Uchida et al., 1983). When there are very large fat globules, it is presumed to be the result of coalescence of smaller droplets and the equivalent of a fat cyst as seen with light microscopy (Schaffner et al., 1963c; Porta et al., 1965; Uchida et al., 1983). Some authors consider that it can occur with participation of adjacent hepatocytes (Schaffner et al., 1963c; Uchida et al., 1983). In agreement with Popper et al., (1981), in this situation, we have found an intervening cellular membrane. Extracellular fat can

also be found within sinusoids (Oudea et al., 1973). Fat observed in portal tracts, is not free as previously assumed, but within the cytoplasmic extensions of macrophages (Popper et al., 1981).

Mallory bodies (MBs) were detected in only a small proportion of those biopsies where they were easily visualised by light microscopy. In only 2 cases were MBs detected at EM and not by light microscopy. MBs seen in this series were composed of filaments with a diameter approximate to that of intermediate filaments. In some cells there was physical proximity or continuity of normal intermediate filaments and MBs. This observation is in keeping with the hypothesis that MBs are generated at least partially from the intermediate filament system (Morton et al., 1980; Fleming et al., 1981; Fleming and McGee, 1984).

In the first description dealing with ALD by Schaffner et al. (1963c), Mallory bodies were thought to be the result of mitochondrial damage. Reppart et al. (1963), described them as large amorphous bodies with the impression that the swollen ER had undergone denaturation to form their protein component. Flax and Tisdale (1964), described Mallory bodies as large irregular, amorphous perinuclear masses without a limiting membrane, and consisting chiefly of irregularly arranged fine granular and filamentous structures. They concluded that Mallory bodies could be related to swollen and degenerated mitochondria as well as altered lysosomes. Biava (1964), confirmed their delicate fibrillar appearance and emphasized that Mallory bodies were not contained within other structures.

Smuckler (1968) and Oudea et al. (1968) insisted on the filamentous nature of the MB meshwork and Smuckler (1968) noted the absence of a true relationship with other organelles. This absence of association with a given organelle was confirmed by Iseri and Gottlieb in 1971. By that time Schaffner et al. (1971) had discarded mitochondria as the source of hyaline and distinguished a fresh fibrillar variety from the more dense lipid-like appearance of older hyaline. They found that cells containing hyaline attracted leucocytes and macrophages. Winckler (1971), in a large series of patients with ALD (mainly with fatty liver), failed to identify intermediate forms between Mallory bodies and mitochondria.

In 1972, Yokoo et al., distinguished three distinct forms of alcoholic hyaline: I) bundles of filaments in parallel arrays, II) clusters of randomly oriented fibrils and, III) a granular or amorphous substance containing only scattered remains of fibrils. Yokoo's group was the first to point out fine filaments in parallel arrangement along the entire circumference of the alcoholic hyaline (mean diameter of 7.4 nm). Similar fine filaments were observed in excess, in cells which did not contain Mallory bodies. Horvath et al. (1973b), in confirming the variability of the structure of hyaline, also referred to three variants, although somewhat different from those of Yokoo et al. Gerber et al. (1973), found that some Mallory bodies too, showed a compact electrodense central mass, surrounded by a loose network of fibrils; the same year, Horvath's group (1973a) confirmed their findings. Ma (1972), reported clumps of ribosomes and polysomes within fibrils.

More recently, French (1981, 1983) published two review articles that deal mainly with the structure, composition and pathogenesis of Mallory bodies. The concept of hyaline material as a pathologic expression of intermediate filament metabolism emerged. French pointed out that the difference between Mallory body filaments and intermediate filaments is in their length, orientation and density; both adhere to organelles, including plasma membranes and nuclei. In addition to MB formation, ALD causes disruption of normal hepatocyte intermediate filament organisation (Barbatis et al., 1986).

Alterations of the sinusoidal wall, including those in the perisinusoidal space of Disse, vary according to the type of ALD. In this study an increase in collagen bundles along the sinusoidal wall was a constant finding in alcoholic hepatitis; this has been recorded by others (Schaffner, 1971; Oudea et al., 1973; Uchida et al., 1983). Since the early descriptions, the extension of collagen between hepatocytes has been recognised (Reppart et al., 1963). In the present study, we have seen a moderate increase in the number of bundles, even in biopsies with minimal histological changes or in instances of mild fatty change.

Schaffner and Popper in 1963, studied 63 patients with chronic liver disease of unspecified type and found that progressive changes in the space of Disse ultimately led to development of a basement membrane and capillarization of sinusoids. Presumably patients with ALD were included, since years later, both Schaffner (1971) and Popper (1981) refer to this finding in ALD. Horvath et al. (1973a) found deposits of basement membrane in most of 30 alcoholic patients studied. Oudea et al. (1968) recognised amorphous material within Disse's space as well as hyperplasia of endothelial cells but no conclusive evidence for capillarization in any of 23 patients studied. A physical basement membrane under sinusoidal cells was rarely seen in the 100 cases of ALD examined in this series.

In alcoholic cirrhosis, widening of the perisinusoidal space is the rule (Schaffner et al., 1963c). Porta et al. (1965) in a study of only 3 alcoholic cirrhotics mentions collagen within Disse's space as well as ceroid pigment within Kupffer cells. Orrego et al. (1979) grading separately the degree of collagenization, the formation of basal membranes and the amount of collagen in the intercellular spaces, established a collagen score. Their results suggested that collagenization of Disse's space may be important in the pathogenesis of ALD. Three years later, the same group confirmed these resuts in a larger sample (111 new cases) (Blendis et al., 1982). In addition, a significant correlation between the intrahepatic interstitial pressure and collagen scores was found.

Lipocytes are mentioned for the first time in relation to ALD in a contribution by Oudea et al. in 1968. They were referred to as "subendothelial cells with fat as seen in rabbits", but they gave no account of significant changes. Takinawa et al. in 1979, referred to fat-storing cells as having proliferated RER with faint flocculent

material in their dilated cisternae, suggesting active protein production, probably protocollagen. The following year Minato et al., (1980) published their results in 20 patients with ALD and were able to group these according to the degree of fibrosis and as a result the authors suggested that lipocytes were associated with fibrogenesis. The concept that lipocytes (perisinusoidal cells) are facultative fibroblasts was advanced earlier on the basis of experimental studies (McGee and Patrick, 1972).

Okanoue et al. (1983), investigated the role of Ito cells in perivenular and intralobular fibrosis of ALD. In fatty liver typical resting Ito cells were seen in the space of Disse. Activated cells with fibroblast-like features were found in areas of hepatocellular degeneration and inflamation, but were absent in cirrhosis without alcoholic hepatitis. Cytoplasmic protrusions of activated Ito cells extended close to hepatocytes containing MBs. Nakano et al. (1982), in a recent study dealing with perivenular fibrosis, found no lipocytes in the connective tissue surrounding the central vein. They were usually located in the midzonal perisinusoidal spaces.

The first contribution on myofibroblasts in ALD was by Rudolph et al. in 1979. In 9 of 12 cirrhotics, there were a high percentage of myofibroblasts among the normal population of fibroblasts. In non-cirrhotic alcoholics, only a small number of fibroblasts contained bundles of microfilaments, although no electrondense bodies or other myofibroblastic features were observed.

Some years later Nakano et al. (1982) evaluated 21 central veins of 11 alcoholics, 4 with fatty liver and 7 with fatty liver and perivenular fibrosis. In both groups, myofibroblasts represented the most common mesenchymal cell. Myofibroblasts surrounded by collagen fibrils were not limited to the perivenular area. Cell processes were seen in the space of Disse associated with netlike fibrosis. Since these cells are considered to synthesize various types of collagen and laminin, the authors suggested that the various sizes of collagen fibrils observed, could be their morphologic counterpart. They concluded that early stages of perivenular fibrosis in alcoholic liver injury are characterised by myofibroblast proliferation. In this study myofibroblasts were rarely observed in fibrous septa and fibrotic portal tracts.

In the literature, changes of the hepatocytic membrane are seldom mentioned. In agreement with some of our findings, on the sinusoidal border, cytoplasmic bullae extending into the space of Disse and flattening of microvilli have beeb found (Rubin and Lieber, 1967; Horvath et al., 1973a; Uchida et al., 1983). Anomalous microvilli on lateral intercellular borders have also been reported, most likely a compensatory change, as seen in chronic conditions other than ALD (Horvath et al., 1973a).

Alterations of bile canaliculi are mentioned in only two reports. Schaffner et al. (1963c), describe dilatation and loss of microvilli. In patients with hyperbilirubinaemia numerous vacuoles can be seen in the pericanalicular zone, most likely lysosomes, as well as vacuoles with a series of rings, presumably bile. Horvath et al. (1973a), found that bile canaliculi did not exhibit marked and consistent changes in all cases.

As in this study, most investigators have had difficulty in identifying Golgi complexes. Early descriptions only mention vacuoles in its vicinity (Schaffner et al., 1963c). Later on, cisternae were described as dilated and containing electrondense granules (Rubin and Lieber, 1967). Ma (1972), found them unremarkable and Horvarth et al. (1973a), described Golgi complexes as dilated and hypertrophied, especially in patients showing disorders of lipid metabolism.

Lysosomal and peroxisomal changes were infrequent in this series. In the literature there is scant reference to lysosomal changes in ALD. Occasionally they are reported as absent (Schaffner et al., 1963c; Porta et al., 1965) while others have found a slight increase in number (Rubin and Lieber, 1967). They may contain iron (Ma, 1972) or in alcoholic hepatitis (Gerber et al., 1973), be engulfing mitochondria. With regard to focal cytoplasmic degradation, information is also limited (Porta et al., 1965; Rubin and Lieber, 1967; Rubin and Lieber, 1968). Ma (1972), prefers the term cytoplasmic lysis and describes it as the replacement of large areas of cytoplasm with electrondense myelin-like strands, appearing as rings, whorls and clumps. The scarcity of data is also applicable to the peroxisomes. They were barely mentioned by Schaffner et al. (1963c) and Porta et al. (1965), referred to structures that suggested dense bodies. Ma (1972), describes them as normal, and later some reports refer to an increase in their number and volume (Horvath et al., 1973a; Oudea et al, 1973). Horvath et al. (1973a), found them within the loose network of Mallory bodies, along with mitochondria, glycogen and other organelles. Sternlieb et al. (1977), in one example of ALD, described slightly enlarged peroxisomes with a predominantly irregular profile and a granular or flocculent matrix. Uchida et al. (1983), found a decrease in the number of peroxisomes in biopsies showing alcoholic foamy degeneration.

In the literature there is scant reference to the amount of glycogen within hepatocytes in ALD. We found great variations both in its amount and distribution, with abundant glycogen in some cells but not in others. Ma (1972), as well as Horvath et al. (1973a), found glycogen to be increased. Horvarth's group observed glycogen to be slightly decreased in those cells with marked SER proliferation. Glycogen bodies were present in 2 of their 30 cases. Uchida et al. (1983), in 5 cases with alcoholic foamy degeneration, estimated as variable the amount of glycogen present.

In summary, the most constant ultrastructural anomalies in ALD are dilatation of ER, mitochondrial size and shape change, fat accumulation, MB formation and collagen deposition. None of these features, however, are invariably present in any single biopsy.

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